EXAMPLE 4

Synthesis of Co[SALEN]

Synthesis of N,N'-bis(salicylidene)ethylenediamine.

To a stirred solution of salicylaldehyde (12.21 g/10.62 mL) in 70°C ethanol (100 mL) was added ethylenediamine (3.01 g/3.33 mL). A yellow crystalline material immediately formed, and the reaction mixture was allowed to cool to room temperature with stirring. The solution was filtered, and the crystals were washed with cold ethanol. The ethanol layers were ombined and reduced to approximately 20 mL and allowed to stand at 0°C overnight. The resulting crystals were collected by vacuum filtration and washed with water. The collected solids were dried *in vacuo* to obtain 13.15 g (98%) N,N'-bis(salicylidene)ethylenediamine as yellow platelets with a melting point of 126°C (literature value (24) 127-128°c). ^{1}H NMR (DMSO-d_6) δ 8.57 (s 2H, HC=N), 7.42 (d, 2H aromatic, J=7.3 Hz), 7.31 (t, 2H, aromatic, J=9.0 Hz), 6.88 (t, 4H, aromatic, J=8.3, 16.1 Hz), 3.89 (s, 4H, CH_2). ^{13}C NMR (DMSO-d_6) δ 166.94 (2C, CO^-), 160.57 (2C, HC+N), 132.38 (2C, aromatic), 131.69 (4C, aromatic), 118.59 (2C, aromatic), 116.51 (2C, aromatic), 58.88 (2C, CH_2).

Synthesis of N,N'-bis(salicylidene)ethylenediaminecobalt(II) (Co[SALEN]).

To a hot (100°C) deoxygenated solution of the above product (2.68 g) in dimethylformamide (25 mL) was added via cannula needle an aqueous solution (10 mL) of cobalt (II) acetate tetrahydrate (2.49 g). The red precipitate which formed was collected by vacuum filtration, washed with cold dimethylformamide, and dried *in vacuo* to obtain 2.6 g (80%) of N,N'-bis-(salicylidene)ethylenediaminocobalt (II) as red crystals.

EXAMPLE 5

Synthesis of Modified Co[SALEN]

The diglycolate ether of Co[SALEN] is prepared as described in Example 4, using the glycolate ether of 2,5-dihydroxybenzaldehyde in place of salicylaldehyde. An unsymmetrically substituted (glycolate ether/amide) complex is prepared as described in Example 4 by using a mixture of the glycolate ether of 2,5-dihydroxybenzaldehyde and 5-aminosalicylaldehyde in place of salicylaldehyde.

EXAMPLE 6

Synthesis of Chlorambucil-Cobalamin Bioconjugates

Synthesis of 1-bromo-2-[4-(4'-[bis-(2-chloroethyl)amino]phenyl)butyroxy]ethane.

Twenty-five mL of freshly distilled CH_2Cl_2, 0.343 g dicyclohexylcarbodiimide (1.66 mmol), 0.305 g 4-dimethylaminopyridine (2.5 mmol), and 0.263 g 4-dimethylamino-pyridinium chloride (1.66 mmol) were added to a flame-dried 50 mL round bottom flask equipped with a stir bar, reflux condenser, and Ar inlet (Boden and Keck, 1985). The solution was purged with argon and brought to reflux. While refluxing, a solution of 0.304 g chlorambucil (1.0 mmol) and 0.125 g 2-bromoethanol (1.0 mmol) in 5 mL CH_2Cl_2 (purged under argon for 30 min.) was transferred via cannula to the refluxing solution over a period of 30 min. After addition was complete, the reaction mixture was stirred for 2 h at room temperature. Precipitated dicyclohexylurea was removed by filtration and the solution was concentrated by rotary evaporation. The resulting residue was taken up in CH_2Cl_2 , filtered, and purified by flash silica column chromatography. The desired product was eluted using 1:9 ethyl acetate:hexanes (v/v) to give 0.374 g of a yellow oil in 91% yield (ester **2**). ^{1}H NMR ($CDCl_3$, 300 MHz) d 7.06 (d, 2H, J=8.4 Hz), 6.60 (d, 2H, J=8.7 Hz), 4.35 (t, 2H, J=6.15 Hz), 3.56-3.72 (m, 8H), 3.48 (t, 2H, J=6.15 Hz), 2.56 (t, 2H, J=7.65 Hz), 2.35 (t, 2H, J=7.35 Hz), 1.91 (quintet, 2H, J=7.58 Hz). ^{13}C NMR ($CDCl_3$, 75 MHz ^{1}H decoupled) d 173.05, 144.35, 130.37, 129.75 (2), 112.12 (2), 63.68, 53.55 (2), 40.60 (2), 33.94, 33.41, 29.05, 26.72.

Synthesis of 2-[4-(4'-[bis-(2-chloroethyl)amino]phenyl)butyroxy]ethylcob(III)alamin (**3**).

Two hundred mg of hydroxocob(III)alamin (0.15 mmol) was dissolved in 10 mL water and purged with Ar while stirring (Brown and Peck, 1988). The exiting gas was conducted in sequence through: (1) a flask containing 0.025 g $NaBH_4$ (0.66 mmol); (2) a flask containing 5 mL H_2O; and (3) a flask containing 0.226 g ester **2** (0.55 mmol) in 5 mL CH_3OH. After deaerating for 1 h, the water from flask (2) was transferred to flask (1) containing $NaBH_4$ via cannula and swirled to promote dissolution. This solution was transferred via cannula to the aqueous cobalamin solution. Reduction was allowed to proceed for 20 min, after which the chlorambucil bromoethylester was added to the solution. The reaction mixture was allowed to stir for an additional 5 min. and then 0.2 mL acetone was added to destroy the excess borohydride. The solution was concentrated to approximately 2 mL by rotary evaporation and

the resulting solution was applied to a 2.5 X 30 cm column of Amberlite XAD-2 resin. The column was washed with 1 L H_2O to desalt and the cobalamin was eluted with 50% aqueous acetonitrile (v/v). The eluent was reduced to approximately 2 mL by rotary evaporation and the solution was applied to a 1 X 40 cm column of SP-Sephadex C25 (Na^+ form). Elution with water removed the major red band which was reduced to a minimal volume. Acetone was added until faint turbidity persisted after swirling. The solution was allowed to stand for 1 h at 0°C and excess acetone was added to promote further crystallization. The crystals were collected by vacuum filtration and dried *in vacuo*. **3** was obtained as red crystals (122.5 mg) with a yield of 53%. MS (FAB+) calcd for $C_{68}H_{112}N_{14}O_{16}CoPCl_2$, 1541; found 1541.

4-[4'-(bis-[2-chloroethyl]amino)phenyl]butyroylcob(III)alamin (**4**) was synthesized in a similar manner starting with the acid chloride of chlorambucil and reating it with hydroxocob(III)alamin as above.

Synthesis of 2-[4-(4'-[bis-(2-chloroethyl)amino]phenyl)butyroxy]ethyl-Co[SALEN] and 4-[4'-(bis-[2-chloroethyl]amino)phenyl]butyroyl-Co[SALEN] are synthsized in a similar manner using Co[SALEN] in place of hydroxocob(III)alamin.

Synthesis of 2-[4-(4'-[bis-(2-chloroethyl)amino]phenyl)butyroxy]ethyl-(Co[SALEN]-folate) and 4-[4'-(bis-[2-chloroethyl]amino)phenyl]butyroyl-(Co[SALEN]-folate) are synthsized in a similar manner using Co[SALEN]-folate in place of hydroxocob(III)alamin.

Synthesis of 2-[4-(4'-[bis-(2-chloroethyl)amino]phenyl)butyroxy]ethyl-(green corrinoid) and 4-[4'-(bis-[2-chloroethyl]amino)phenyl]butyroyl-(green corrinoid) are synthsized in a similar manner using CH_3-Co(III) corrinoid (prepared by reacting methyliodide with the green corrinoid of Brown et al. (1996) after it had been reduced with $NaBH_4$) in place of hydroxocob(III)alamin.

EXAMPLE 7

Sonolysis of 2-[4-(4'-[bis-(2-chloroethyl)amino]phenyl)butyroxy]ethylcob(III)alamin (**3**)

The products released by exhaustive sonolysis, as described in Example 2, of compound **3** (prepared in Example 6) were isolated by reverse-phase HPLC (Rainin Microsorb C-18). Elution and separation of the sonolysis products were carried out with an increasing gradient of acetonitrile (A) and 0.05 M phosphoric acid (B): initial condition 5% A: 95% B, increased linearly for 10 min to 30% A and 70% B, maintained for 2 min; followed by a linear increase to